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Involvement of GPR30 in protection effect of Dexmedetomidine against myocardial ischemia/reperfusion injury in rat *via* AKT pathway

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Acute myocardial infarction (AMI) is a heart disease that seriously threatens human health. Dexmedetomidine (DEX) has a certain protective effect on cardiac injury. This study investigated the cardioprotective effect of DEX and its potential molecular mechanism *in vivo* and *in vitro*. The results showed that DEX could significantly increase the viability of hypoxia/reoxygenation (H/R) treated cardiomyocytes and reduce oxidative damage and apoptosis. Further molecular mechanism analysis showed that the above cardiac protective effects may be related to Akt signaling pathway. In addition, the expression of G-Protein Receptor 30 (GPR30) was promoted after H/R treatment. However, knockdown of GPR30 by shRNA significantly counteracted the cardioprotective effect of DEX. Meanwhile, we constructed a rat model of AMI to investigate the role of GPR30 *in vivo*. The results showed that DEX significantly reduced the infarct size, and GPR30 agonist G1 enhanced the protective effect of DEX on heart. On the contrary, protein kinase B (AKT) inhibitor LY294002 counteracted the protective effect of DEX on heart, suggesting that GPR30 enhanced the protective effect of DEX on ischemia-reperfusion induced heart injury by regulating AKT related pathways. In conclusion, our study provides a potential target for the clinical treatment of AMI.

Keywords: acute myocardial infarction, Dexmedetomidine, G-protein receptor 30, myocardial ischemia/reperfusion injury, AKT pathway

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Abbreviations: AMI, acute myocardial infarction; DEX, Dexmedetomidine; H/R, hypoxia/reoxygenation; GPR30, G-Protein Receptor 30; AKT, protein kinase B; I/R, ischemia-reperfusion; PI3K, Phosphatidylinositol 3 kinase; ADCA, anterior descending coronary artery; AAR, area at risk; PBS, phosphate buffered solution; DMEM-F12, Dulbecco's Modified Eagle Media: Nutrient Mixture F-12; FBS, fatal bovine serum; 5-BrdU, 5-bromodeoxyuridine; MTT, 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide; DMSO, dimethyl sulfoxide; OD, Optical density; SOD, superoxide dismutase; MDA, Malondialdehyde; RT-qPCR, Real time quantitative PCR; GAPDH, glyceraldehyde-3-phosphate dehydrogenase; RIPA, Radioimmunoprecipitation assay; SDS–PAGE, sodium dodecyl sulfate polyacrylamide gel electrophoresis; PVDF, polyvinylidene fluoride; siRNA, small interfering RNA; S.D., standard deviation; shRNA, short hairpin RNA; LDH, lactic dehydrogenase; SIRT1, silence information regulator1; mTOR, mammalian target of rapamycin; Erα, estrogen receptor alpha; Erβ, estrogen receptor beta; EGFR, epidermal growth factor receptor; MAPK, mitogen activated protein kinase; PKA, protein kinase A; OVX, ovariectomized

INTRODUCTION

Acute myocardial infarction (AMI) is a heart disease that seriously threatens human health. With the change of people's lifestyle and the aging of the population, cardiovascular diseases, especially congestive heart failure and malignant arrhythmias caused by myocardial infarction, have a very high morbidity and mortality all over the world (Lloyd-Jones *et al.,* 2010; JENKO *et al.,* 2019). In recent years, with the extensive development of thrombolysis and cardiac interventional surgery and the rapid development of drug therapy in the treatment of myocardial infarction, the prognosis of patients with myocardial infarction has been greatly improved, but there are still many patients who failed to carry out revascularization in time for various reasons. The irreversible death of myocardium leads to ventricular remodeling, which leads to deterioration of cardiac function and eventually to heart failure (Eapen *et al.,* 2012).

Dexmedetomidine (DEX) is a novel highly selective α 2-adrenergic receptor agonist, which is widely used in intensive care unit and clinical anesthesia (Eltzschig & Eckle, 2011). Studies have found that DEX has a protective effect on lung, kidney and other organ injury, and can reduce apoptosis and inhibit inflammatory response (Vincent *et al.,* 2013; Lin & Knowlton, 2014). In recent years, a number of studies have shown that DEX has a certain protective effect on cardiac injury, including reducing myocardial ischemia-reperfusion (I/R) injury, stabilizing heart rhythm, and reducing the incidence of complications of cardiac surgery (Peng *et al.,* 2013; Xu *et al.,* 2013; Chen *et al.,* 2014) by regulating antioxidant and anti-inflammatory signals (Wang *et al.,* 2020). However, the detailed mechanism still needs to be further explored.

G-Protein Receptor 30 (GPR30) is an estrogen receptor which plays an important role in the protection of myocardium against myocardial injury induced by I/R. It has been found that GPR30 agonist treatment can significantly reduce isolated myocardial I/R injury in male rats (Deschamps & Murphy, 2009; Bopassa *et al.,* 2010). The activation of GPR30 promoted the recovery of rat cardiac function and reduced myocardial inflammation by increasing cell viability and inhibiting apoptosis (Weil *et al.,* 2010). Our pre-experimental studies showed that DEX preconditioning increased the expression of GPR30 in I/R myocardium, so we speculate that the cardioprotective effect of DEX may be achieved through GPR30.

Phosphatidylinositol 3 kinase (PI3K)-protein kinase B (AKT) signaling pathway plays an important role in various diseases (Gu *et al.,* 2020), including myocardial I/R

injury (Yang *et al.,* 2004). In I/R, it was initially found to be associated with pathways, such as mitochondrial dysfunction, oxygen free radical production, neutrophil aggregation, calcium overload etc. Further studies have shown that the protective mechanism in cardiomyocytes has played a role before serious consequences, and one of the important mechanisms is the PI3K-AKT signal pathway (Yang *et al.,* 2005). Interestingly, Wei et al found that the activation of GPR30 may provide cardiac protection through downstream activation of PI3K-dependent pathways in I/R (Deschamps & Murphy, 2009). Therefore, the current study investigated the role of GPR30 in the protection of DEX against myocardial I/R injury in rats and its potential molecular mechanisms.

MATERIAL AND METHODS

Animals and Experimental protocols

Animal experiments were approved by the Ethics Committee of Experimental Animals in Medical College of Jiaxing University and carried out in accordance with the National Institutes of Health Guide for the Care and Use of Laboratory Animals. Male Sprague–Dawley rats (300 g) were purchased from Shanghai Experimental Animal Center (Shanghai, China), and given humane care. Rats were anesthetized by intraperitoneal injection of pentobarbital sodium (80 mg/kg) and mechanical ventilation. The left chest was opened through the fourth intercostal space and the heart was revealed by pericardiotomy. To evaluate transient ischemia of the left ventricular, 6-0 Prolene ring was placed at the site of the first branch of the anterior descending coronary artery (ADCA), and the end of the suture passed through PE-50 to form a snare for reversible occlusion. Then, the heart was subjected to regional ischemia for 30 min, followed by reperfusion of area at risk (AAR) (ADCA dependent area) for 120 min. Sixty rats were randomly divided into 6 groups with 10 rats in each group: Sham group, AMI group (rats were given equal amount of normal saline before modeling), AMI+DEX group (rats were given 1 μg/kg/d DEX by intravenous injection for 30 min), AMI+DEX+G1 group (rats were given 1 μg/kg/d DEX and 100 nM G1 by intravenous injection for 30 min before modeling), AMI+LY294002 group (rats were given 1 μg/kg/d DEX and 20 μM G1 by intravenous injection before modeling) and AMI+DEX+G1+LY294002 group (rats were given 1 μg/kg/d DEX, 100 nM G1 and 20 μM LY294002 by intravenous injection for 30 min before modeling). After 10 weeks, animals were euthanized by intraperitoneal injection of 200 mg/kg pentobarbital sodium.

Isolation and treatment of primary cardiomyocytes

Primary cardiomyocytes were isolated as previously described (Gao & Meng 2017). In short, heart of neonatal rats (1–2 days old) was taken out and maintained in cold phosphate buffered solution (PBS). Ventricle was cut into small pieces (1~3 mm^3) and digested with 0.1% type II collagenase at 37°C for 5 min, repeated five times. The supernatant was collected by centrifugation and resuspended with Dulbecco's Modified Eagle Media: Nutrient Mixture F-12 (DMEM-F12) containing 15% fatal bovine serum (FBS) (C11330500 ETQ Gibco). Separate fibroblasts and cardiomyocytes by differential wall method, and the growth of fibroblasts was inhibited by 5-bromodeoxyuridine (5-BrdU) (B5002 pr. Louisjue USA). For *in vitro* hypoxia/reoxygenation (H/R) model, cells were pre-treated with different doses of DEX (0.1, 0.5, 1 and 5 μM), and then subjected to $Na_2S_2O_4$ (4 mM) at 37°C for 1 h. Thereafter, the cells were cultured with normal medium for another 12 h to generate a reoxygenated wcondition.

MTT assay

Cell viability was monitored by 3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide (MTT) assay. In brief, cardiomyocytes were exposed to MTT (50 μM) at 37°C for 4 h. Thereafter, the medium was carefully removed and 100 μl of dimethyl sulfoxide (DMSO) was added. Optical density (OD) values of formazan crystals were determined at 570 nm using microplate reader (BioTeck, Winooski, Vermont, USA).

Measurement of SOD and MDA

Enzymatic activity of superoxide dismutase (SOD) was detected using Total Superoxide Dismutase Assay Kit with NBT (S0109, Beyotime, Shanghai, China), while Malondialdehyde (MDA) were detected using Lipid Peroxidation MDA Assay Kit (S0131S, Beyotime, Shanghai, China) as per manufacturer's instructions

Real time quantitative PCR (RT-qPCR)

Total RNA from cells was isolated and reversely transcribed using FastKing gDNA Dispelling RT SuperMix (Tiangen, Beijing, China). The expression of GPR30 was measured by RT-qPCR using Quant one step qRT-PCR Kit (SYBR Green, FP303, Tiangen, Beijing, China) in a Mastercycler EP realplex detection system (Roche, Indianapolis, IN). The expression of GPR30 was normalized by glyceraldehyde-3-phosphate dehydrogenase (GAPDH), and analyzed using $2^{-\Delta\Delta CT}$ method (Schmittgen & Livak, 2008). Primers were as follows:

GPR30, F, 5'-GACCTGATATTGACCTTG-3', R, 5'-CCACAATATCGACTTCAATC-3'.

GAPDH, F, 5'-AACTGAACCTGACCAACG-3', R, 5'-TTCAAGGCTGCATGCCAAC-3'.

Western blotting

Proteins in infarcted heart tissue or cells were extracted using Radioimmunoprecipitation assay (RIPA) lysis buffer (Beyotime, Shanghai, China) with phosphatase inhibitors (Abcam, Cambridge, UK). Protein levels were measured by Western blotting. In brief, protein (20 μg) was separated by 10% sodium dodecyl sulfate polyacrylamide gel electrophoresis (SDS–PAGE) and then transferred on to polyvinylidene fluoride (PVDF) membranes (Roche, Switzerland). The membranes were incubated with primary antibodies, such as anti-GPR30 (ab39742, 1:250, Abcam, Cambridge, UK), anti-Bcl2 (ab32124, 1:1000, Abcam, Cambridge, UK), anti-cleaved caspase 3 (ab32042, 1:500, Abcam, Cambridge, UK), anti-caspase 3 (ab13847, 1:500, Abcam, Cambridge, UK), anti-AKT (ab38449, 1:500, Abcam, Cambridge, UK), anti-p-GSK-3β (ab93926, 1:500, Abcam, Cambridge, UK), anti-GSK-3β (ab32391, 1:5000, Abcam, Cambridge, UK), anti-β-actin (ab8227, 1:1000, Abcam, Cambridge, UK) at 4°C overnight, and incubated with secondary antibody Goat Anti-Rabbit IgG H&L (HRP) secondary antibody (ab6721, 1:2000; Abcam, Cambridge, UK) for 1 h at room temperature. Blots were visualized using a FluroChem E Imager (ProteinSimple, Santa Clara, CA, USA) and protein levels were quantified with Quantity

AlphaEaseFCTM (Alpha Innotech, San Leandro, CA, USA) imaging software.

GPR30 knockdown

To investigate the effect of GPR30 *in vitro*. The cells were infected with adenovirus vectors (VectorBuilder, Guangzhou, China) containing shGPR30 fragments for GPR30 silencing (shGPR30#1 or shGPR30#2) or shRNA as negative control. Sh-GPR30 was designed by annealing two pairs of small interfering RNA (siRNA) fragments. The sequences were as follows:

ShRNA1# (i) 5'-AAGTGGCTTCGTACATAACGTC-CTGTCTC-3' (sense), and 5'-AAGCCTACCAATGTAAC-TACGCCTGTCTC-3' (antisense); (ii) 5'-AACCAAAT-GCTAGGAACTGCACCTGTCTC-3' (sense) and 5'-AACAACCAAACTGCCTCTGAACCTGTCTC-3' (antisense).
ShRNA2# (i) 5'-AAGCCGGTAAACTGACAAATGCCT-GTCTC-3' (sense), and 5'-AAGTTGCTAGCTCAAATC-GAGCCTGTCTC-3' (antisense); (ii) 5'-AACCTAGCTGFAT-GCCTGGACCCTGTCTC-3' (sense) and 5'-AACCGTAGCT-TAGTGCCATGCCCTGTCTC-3' (antisense)

Triphenyl tetrazolium chloride (TTC) staining

Myocardial infarction was detected by TTC staining. At the end of the reperfusion, hearts were quickly removed from mice and stored at –80°C. The myocardial tissue was sectioned (2 mm), and then incubated with 1% TTC solution (sigma Aldrich) at 37°C for 30 min. Kept away from light for 30 min. After washing and fixing, the photos were taken and analyzed by Image-Pro Plus6.0 software.

Statistical analysis

All data are presented as mean standard deviation (S.D.) Unpaired t-test was used for the difference between two groups, while one-way ANOVA followed by Bonferroni test was used for the difference between multiple groups. $p<0.05$ was considered statistically significant. GraphPad Prism 5 was used for statistical analysis.

RESULTS

DEX up-regulated the expression of GPR30, which increased cell viability and reduced oxidative damage of cardiomyocytes treated with H/R

This study investigated the effect of DEX on the viability of cardiomyocytes. Cell viability was detected by MTT assay. As shown in Fig. 1A, different doses of DEX had little effect on the viability of cardiomyocytes. However, after the cardiomyocytes were treated with H/R, DEX had a significant effect on the viability of the cardiomyocytes. As shown in Fig. 1B, compared with the control group, H/R treatment significantly reduced the viability of cardiomyocytes. Notably, DEX (1 μM) reversed the inhibitory effect of H/R on cardiomyocyte viability. Compared with 1 μM, when the dose of DEX was 5 μM, the reversal effects of DEX were weakened. Besides, our study also showed that H/R treatment reduced the activity of SOD and increased the content of MDA. Interestingly, DEX (1 μM) reversed the reduction of SOD activity and the increase of MDA content by H/R. In addition to 1 μM of DEX, 0.5 μM of DEX also showed a reversal effect on the increase of MDA content. Compared with 1 μM, when the dose of DEX was 5 μM, the effects of DEX were weakened (Fig. 1C–D). Further analysis showed that different doses of DEX promoted the expression of GPR30 at the mRNA and protein level in a dose-dependent manner, which further promoted the expression of anti-apoptosis-related protein (Bcl2) and inhibited the expression of apoptosis-related protein (Cleaved caspase 3) (Fig. 1E–F). In summary, our findings demonstrated that DEX up-regulated the expression of GPR30, which increased cell viability and reduced oxidative damage of cardiomyocytes treated with H/R.

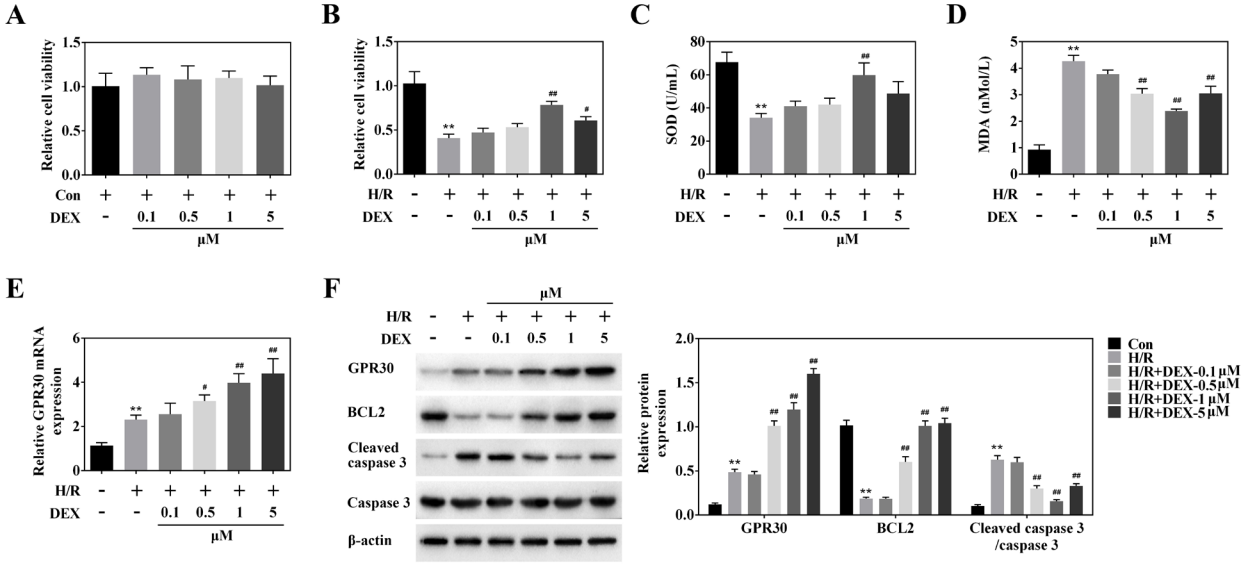

Figure 1. DEX up-regulated the expression of GPR30 and increased the cell viability of cardiomyocytes treated with H/R. Cardiomyocytes or H/R cardiomyocytes were pre-treated with different doses of DEX (0, 0.1, 0.5, 1 and 5 μM) for 1 h. (**A–B**) Cell viability was measured by MTT assay. (**C**) SOD. (**D**) MDA. (**E**) The expression of GPR30 was measured by qPCR. F. The protein levels of GPR30, Bcl-2, Cleaved caspase 3, Caspase 3 were measured by western blotting. ($^{**}p<0.01$ *vs* Control group, $^{\#}p<0.05$, $^{\#\#}p<0.01$ *vs* H/R group)

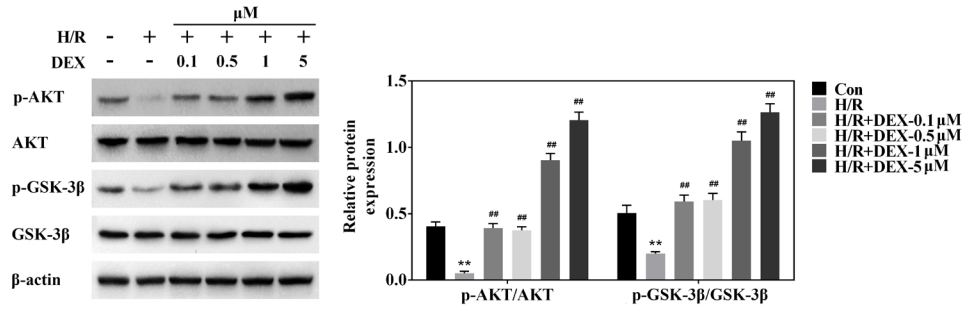

Figure 2. DEX activated the AKT signal pathway. H/R cardiomyocytes were pre-treated with different doses of DEX (0, 0.1, 0.5, 1 and 5 μM) for 1 h.
The protein levels of p-AKT, AKT, p-GSK-3β and GSK-3β were measured by western blotting. (**p<0.01 *vs* Control group, #p<0.05, ##p<0.01 *vs* H/R group)

DEX activated the AKT signal pathway

PI3K-AKT signaling pathway plays a very important role in myocardial I/R injury. Therefore, this study explored the effect of DEX on AKT signal pathway. As shown in Fig. 2, the level of phosphorylation of AKT in cardiomyocytes treated with H/R was significantly lower than that of the control group, followed by a decrease in the level of phosphorylation of GSK-3β, a protein downstream of AKT. Interest-

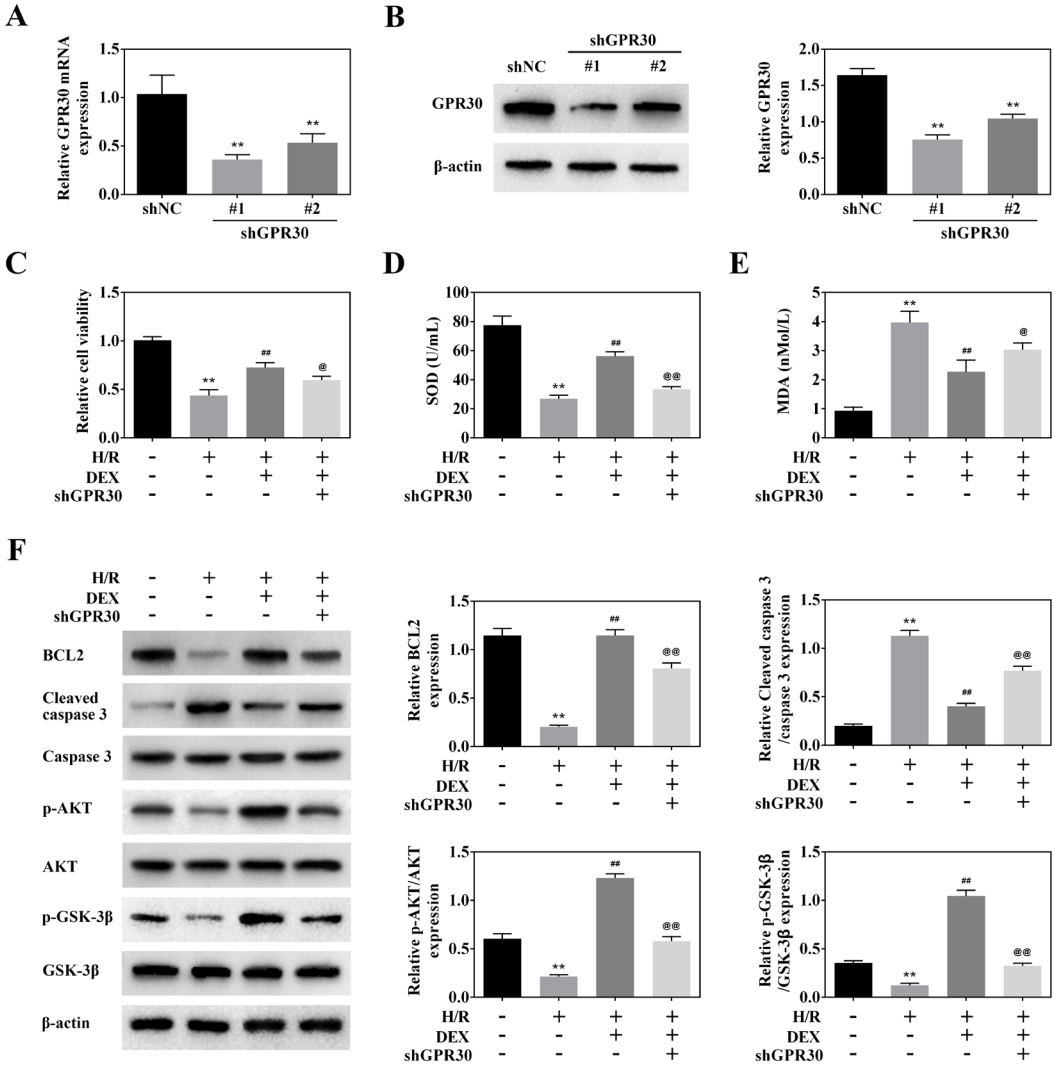

Figure 3. GPR30 knockdown reversed the protective effect of DEX on cardiomyocytes treated with H/R.
(**A–B**) GPR30 was knocked down using shGPR30#1 or shGPR30#2. A. The expression of GPR30 was measured by qPCR. B. The protein level of GPR30 was measured by western blotting. (**p<0.01 vs shNC group). (**C–F**) H/R cardiomyocytes were pre-treated with DEX at a dose of 1 μM for 1 h or/and transfected with shGPR30#1. C. Cell viability was measured by MTT assay. D. SOD. E. MDA. F. The protein levels of Bcl-2, Cleaved caspase 3, Caspase 3, p-AKT, AKT, p-GSK-3β and GSK-3β were measured by western blotting. (**p<0.01 *vs* Control group, #p<0.05, ##p<0.01 *vs* H/R group, @p<0.05, @@p<0.01 *vs* H/R+DEX group)

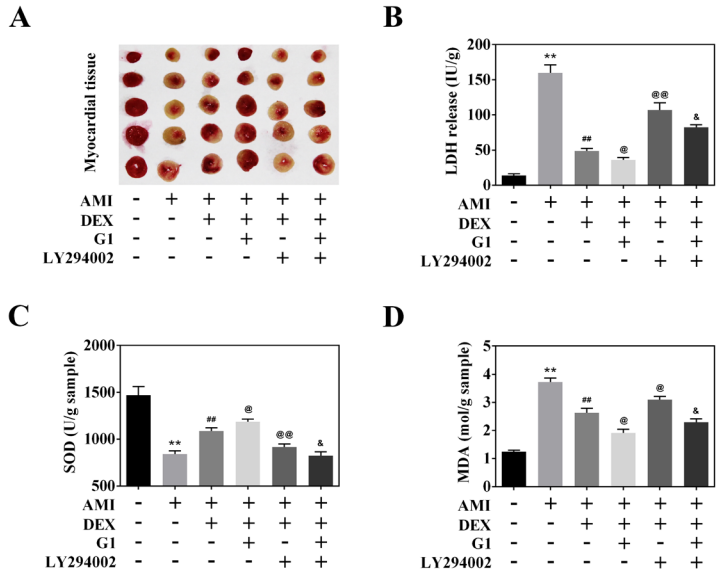

Figure 4. GPR30/AKT signal pathway plays a role in myocardial protection of DEX.
Sixty rats were randomly divided into 6 groups with 10 rats in each group: Sham group, AMI group (rats were given equal amount of normal saline before modeling), AMI+DEX group (rats were given 1 μg/kg/d DEX for 30 days), AMI+DEX+G1 group (rats were given 1 μg/kg/d DEX and 100 nM G1 for 30 days before modeling), AMI+LY294002 group (rats were given 1 μg/kg/d DEX and 20 μM G1 before modeling) and AMI+DEX+G1+LY294002 group (rats were given 1 μg/kg/d DEX, 100 nM G1 and 20 μM LY294002 for 30 days before modeling). (**A**) TTC staining was used to detect myocardial infarction. (**B**) LDH in tissue. (**E**) SOD in tissue. (**F**) MDA in tissue. ($^{**}p<0.01$ *vs* Control group, $^{\#}p<0.01$ *vs* AMI group, $^{@}p<0.05$ *vs* AMI+DEX group, $^{\&}p<0.05$ *vs* AMI+DEX LY294002 group)

ingly, DEX significantly reversed the inactivation of AKT and its downstream protein GSK-3β in cardiomyocytes induced by H/R in a dose-dependent manner. All in all, our results showed that DEX activated the AKT signal pathway.

GPR30 knockdown reversed the protective effect of DEX on cardiomyocytes treated with H/R

To clarify whether the high expression of GPR30 is involved in the protective effect of DEX on cardiomyocytes, we first use short hairpin RNA (shRNA) to knock down GPR30. As shown in Fig. 3A–B, after knockdown of GPR30 by shGPR30#1 or shGPR30#2, the expression of GPR30 in cardiomyocytes was significantly inhibited, and the knockdown effect of shGPR30#1 was better than that of shGPR30#2. Therefore, the follow-up experiment was carried out by using shGPR30#1 with high knockout efficiency. Then we investigated the effect of low expression of GPR30 on cardiomyocytes treated with H/R. As shown in Fig. 3C, DEX significantly reversed the decrease in cardiomyocyte viability after H/R treatment, which was offset by GPR30 knockdown. Besides, GPR30 knockdown also offset the increase of SOD activity and the decrease of MDA content in H/R cardiomyocytes pretreated with DEX (Fig. 3D–E). In addition, GPR30 knockdown offset the increase of Bcl-2 and the decrease of cleaved caspase-3 in H/R cardiomyocytes pretreated with DEX. Further analysis showed that GPR30 knockdown reversed the activation of the AKT–GSK-3β pathway by DEX (Fig. 3F). Overall, our results showed that GPR30 knockdown reversed the protective effect of DEX on cardiomyocytes treated with H/R.

GPR30/AKT signal pathway plays a role in myocardial protection of DEX

As a supplement, we also confirmed the effect of GPR30 on myocardial protection induced by DEX *in vivo*. As shown in Fig. 4A, TTC staining showed that AMI rats had severe myocardial infarction compared with the sham group, and the degree of myocardial infarction in AMI rats was improved after DEX treatment. Compared with DEX alone, myocardial infarction was significantly improved in AMI rats treated with DEX and GPR30 agonist (G1). It is worth noting that compared with DEX alone, myocardial infarction was significantly aggravated in AMI rats treated with DEX and AKT inhibitor (LY294002). Besides, we detected the levels of lactic dehydrogenase (LDH) and MDA, as well as the activity of SOD in myocardial tissue of rats in each group. As shown in Fig. 4B–D, LDH and MDA in myocardium of AMI rats increased significantly, while SOD activity significantly decreased. After DEX treatment, the levels of LDH and MDA decreased significantly, while the activity of SOD increased. More importantly, compared with DEX alone, when AMI rats were treated with DEX and GPR30 agonist (G1), the levels of LDH and MDA were further decreased, while the activity of SOD was further increased. However, compared with DEX alone, when AMI rats were treated with DEX and AKT inhibitor (LY294002), the levels of LDH and MDA increased again, while the activity of SOD decreased as well. Taken together, these results suggest that GPR30/AKT signal pathway plays a role in myocardial protection of DEX.

DISCUSSION

Ischemic heart disease remains the leading cause of global deaths, and its persistence is the main cause of high mortality and incidence rate worldwide (Mendis *et al.,* 2015). Although great progress has been made in the treatment of ischemic heart disease, the injury caused by I/R still limits the recovery of myocardial injury and acute myocardial infarction (Spath *et al.,* 2016). In patients with AMI, antiplatelet, antithrombotic therapy, revascularization and drug therapy such as blocking, statins

and renin angiotensin aldosterone axis inhibitors improve cardiac remodeling and subsequent cardiac events, including myocardial ischemia and cardiac exhaustion (Hamm *et al.,* 2011; Ibanez *et al.,* 2018; Neumann *et al.,* 2019).

DEX has the effects of sedation, analgesia and opioids, and is a routine perioperative drug, especially for short-term and long-term sedation in intensive care patients (Keating, 2015). Previous studies have shown that DEX has obvious cardioprotective effects on myocardial I/R rats (Cheng *et al.,* 2016; Behmenburg *et al.,* 2017; Bunte *et al.,* 2020). For example, Tang and others (Tang *et al.,* 2020) found that DEX preconditioning alleviated acute myocardial infarction, oxidative stress and myocardial injury after ischemia by regulating endoplasmic reticulum stress and reducing cell injury, thus achieving cardioprotective effect. Besides, Zhang and others (Zhang *et al.,* 2020) found that DEX protected myocardium by upregulating silence information regulator1 (SIRT1)/ mammalian target of rapamycin (mTOR) axis and reducing excessive autophagy to reduce cardiomyocyte apoptosis, oxidative stress and inflammation. Similarly, this study confirmed that DEX has a cardioprotective effect by elevating the vitality of H/R cardiomyocytes and inhibiting their apoptosis, reducing oxidative damage. Moreover, after treating AMI rat models with different doses of DEX, we found that DEX can reduce the area of myocardial infarction and reduce oxidative damage. Further mechanism analysis showed that the cardioprotective effect of DEX may be related to the AKT signaling pathway.

Estrogen is a steroid hormone with a wide range of biological activities. It is generally believed that estrogen plays a role through the downstream signaling pathway mediated by estrogen receptor alpha (ERα) and estrogen receptor beta (Erβ), but the activation of these pathways can lead to side effects such as breast cancer and endometrial hyperplasia (Robinson *et al.,* 2013). In recent years, it has been found that GPR30, a subtype of ER located on cell membrane, has an important cardioprotective effect (Weil *et al.,* 2010). Estrogen has high affinity for GPR30 and could induce rapid signal transduction through GPR30 and epidermal growth factor receptor (EGFR), including activation of mitogen activated protein kinase (MAPK), protein kinase A (PKA) and phosphatidylinositol 3 kinase (PI3K) (Prossnitz & Maggiolini 2009). Besides, Zhu and others (Zhu *et al.,* 2020) found that GPR30 and its upstream regulatory genes, miR-2861 and miR-5115, were differentially expressed in myocardial I/R by microarray analysis of GES67308 and GES50885, in which the expression of GPR30 was suppressed, and miR-2861 and miR-5115 inhibited the expression of GPR30. Notably, overexpression of GPR30 alleviated pathological damage, myocardial infarction and apoptosis in mice. Moreover, GPR30 specific agonist G1 reduces I/R-induced myocardial infarction by reducing myocardial inflammation, improving immune suppression, and triggering a pro-survival signal cascade (De Francesco *et al.,* 2017). On this basis, this study found the high expression of GPR30 in H/R cardiomyocytes, while DEX treatment could promote the expression of GPR30, suggesting that the myocardial injury induced by H/R may be related to the low expression of GPR30, and the cardioprotective effect of DEX may be related to GPR30. So, we designed shRNA to knock down GPR30. Surprisingly, knockdown of GPR30 reversed the increase of cardiomyocyte viability after DEX treatment, aggravated oxidative damage and accelerated cardiomyocyte apoptosis. On the contrary, *in vivo* studies have shown that GPR30 agonist G1 significantly enhanced the ameliorative effect of DEX on myocardial infarction. Therefore, this study found for the first time that GPR30 participates in the protective effect of DEX on myocardial I/R injury.

PI3K-AKT pathway is an important pathway in the process of myocardial I/R injury (Xin *et al.,* 2020). *In vivo* and *in vitro* studies found that regulation of PI3K/Akt/mTOR signaling pathway inhibited autophagy of cardiomyocytes, thereby reducing myocardial I/R injury (Qiu *et al.,* 2020). Previous studies have shown that activating GPR30 may regulate cell viability, apoptosis and inflammation by activating PI3K-dependent pathways, thereby alleviating heart injury induced by I/R (Deschamps & Murphy, 2009). Some other studies have shown that GPR30 reduced myocardial infarction and fibrosis in female ovariectomized (OVX) mice by activating PI3K/AKT pathway (Wang *et al.,* 2019). In addition, Chang et al investigated the potential molecular mechanism of DEX on myocardial I/R injury, and found that preconditioning may activate PI3K/AKT signal pathway by relying on α-adrenoceptor, and further confirmed that DEX preconditioning may inhibit Imax R-induced apoptosis by activating PI3K/Akt signal pathway, thus has a cardioprotective effect on myocardial I/R injury in diabetic rats (Chang *et al.,* 2020). Consistent with the above study, this study also found that DEX protected the heart by regulating AKT-related signaling pathways. Curiously, this study found that GPR30 participated in this process and enhanced the protective effect of DEX on myocardial I/R injury by enhancing the activation of AKT pathway.

In conclusion, in this study we investigated the cardioprotective effect of DEX and its potential molecular mechanism *in vitro* and *in vivo*. The results showed that DEX increased the viability of H/R cardiomyocytes and reduced apoptosis and myocardial infarction area by regulating AKT pathway. Further study found that GPR30 was involved in the protective effect of DEX on H/R and AMI. Our study provides a potential target for the clinical treatment of AMI.

Acknowledgements

Not applicable.

Competing interests

The authors state that there are no conflicts of interest to disclose.

Ethics approval

Ethical approval was obtained from the Ethics Committee of Experimental Animals in Medical College of Jiaxing University.

Statement of Informed Consent

Written informed consent was obtained from a legally authorized representative(s) for anonymized patient information to be published in this article.

Availability of data and materials

All data generated or analyzed during this study are included in this published article.

Authors' contributions

Zheming Shao and Qihong Shen designed the study, supervised the data collection, Min Kong and Huadong Ni analyzed the data, interpreted the data, Xiaomin Hou

prepare the manuscript for publication and reviewed the draft of the manuscript. All authors have read and approved the manuscript.

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